Biological aspects of green mussels *Perna viridis* cultivated on raft culture in Pasaran coastal waters, Indonesia

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Abstract. The green mussel (*Perna viridis*) is widely distributed in Indonesia. In Lampung province, the *P. viridis* is cultivated on floating raft system surrounding the coastal water of Pasaran Island. *P. viridis* meat is one of the cheap and important protein sources from marine resources. However, information on the cultivation and some biological aspects of *P. viridis* from Pasaran Island has not reported yet, even though this commodity has been cultivated since 2012. This study aims the monitoring of the growth performance, body condition index (BCI) and gonadal profiles of the *P. viridis* cultivated from Pasaran Island. Differentiation of male and female specimens is quite simple, by only observing the color of mussel meat directly. Females are reddish while males are gray to brown. The traditional fisherman used to harvest the mussels after 6-8 months cultivation. The growth rate of males and females did not showed significant difference but BCI values. The differences among ages affected the total weight, BCI, meat weight, shell length, thickness, width, and weight ratio. Shell length and weighing of males is 55.7 mm and 10.38 g respectively, at the age of 6 months, while in females 57.3 mm and 9.82 g respectively. The female BCI value is double comparing to males, after six months of cultivation (1.63 and 0.84). Gonads begin to be found at the age of 3 months with gonadal maturity starting at the age of 4-6 months.

Key Words: growth, BCI, gonad, morphometric, sex determination.

Introduction. The green mussel (*Perna viridis*) worldwide is distributed in the Indo-Pacific region, extending from Japan to New Guinea and from the Persian Gulf to South Pacific islands (Tan & Ransangan 2016). In tropical countries such Indonesia, *P. viridis* are widely distributed on almost all coastal regions of the Indonesian islands, dispersed from Sumatera in Malacca Strait, Lampung Strait, Sunda Strait, while in Java island located in Lada bay, Jakarta Bay, Java sea, and Indian ocean. In eastern Indonesia is found in Nusa Tenggara coastal waters, Makassar Strait to Ambon Bay (Evans et al 1995; Sudharyanto et al 2005; Arfin et al 2012; Yaqin et al 2011; Huhn et al 2015).

According to Davy & Graham (1982), *P. viridis* farming in Indonesia began in the late 1970s with the first cultivation carried out in Jakarta and Banten Bay. Followed by cultivation in Belawan, North Sumatera and Surabaya, Java Sea (Sudharyanto et al 2005), and Lampung Strait (Noor 2015).

In Indonesia, the meat of *P. viridis* is accustomed for human consumption and it is an important species in aquaculture. Also, they were fast-growing and inexpensive protein source (Rajagopal et al 2006). Green mussels generally lived near to the estuary, found attached to wood, bamboo, coral, ropes as their substrate through the byssus (Yonvitner & Sukimin 2009). The seeds are naturally attached to collectors (Mohamed 2015), and ready to harvest after 6-7 months of cultivation (Noor 2015).

The cultivation of *P. viridis* in Lampung strait is performed in the coastal waters of Pasaran Island which is located in the west of Teluk Betung district, Bandar Lampung City. According to Ali et al (2015), this location is an adequate environment, with carrying capacity to cultivate *P. viridis*, since these waters have low currents, and there

are naturally available *P. viridis* seeds. The cultivation of *P. viridis* adopts a raft culture system due to its ease management (Noor 2015).

Biological characteristics of the *P. viridis* in Lampung strait have not been reported yet, as well as growth performance, morphometric, condition index performance, and gonad index, having significant contribution in developing the future culture. This study aims to monitor the biological aspects of *P. viridis* cultivated on raft system in Pasaran Island waters.

Material and Method

Study area and culture condition. The research location was around the Pasaran island coastal waters (S050 27'54 524", E1050 15'39 468") in Lampung bay of Indonesia (Figure 1). The mussel samples were obtained from a floating raft culture within raft dimension of 10 x 10 m (Sulvina et al 2015).

The substrate used for *P. viridis* seedling consisted of 200 cm of ropes in length and made from natural fibers. The ropes were tied to bamboo cross the surface of the seawater and at the end of the ropes ballasts were tied. After 1-3 weeks from the installation, the *P. viridis* seeds was tapped on the substrate and harvested at the age of 5-6 months (Noor 2015; Noor et al 2016).

Figure 1. Map and the mussel farming in Pasaran Island, Indonesia.

P. viridis sampling. Sample specimens were harvested in the morning, and then washed and weighed, then followed by classifying of sexes differentiation of *P. viridis* based on monitoring the different coloration of the tissue and gonad (Arshad 2012); whereas the age determination was based on the measurement of the shell length and information's from the local fisherman.

Morphometric analysis. Morphometric analysis was conducted by weighing the total and tissues weight followed by measuring the shell dimension by caliper, including total length, width, and height (Figure 2), followed by identification of height ratio, width ratio, and weight ratio.

$$Breadth\ ratio = \frac{av.heigth\ (b)}{av.length\ (L)}$$

$$Width\ ratio = \frac{av.width\ (w)}{av.lenght\ (L)}$$

$$Weigh\ ratio = \frac{av.weight\ of\ meat\ (Wm)}{av.of\ total\ weigh\ (Tw)}$$

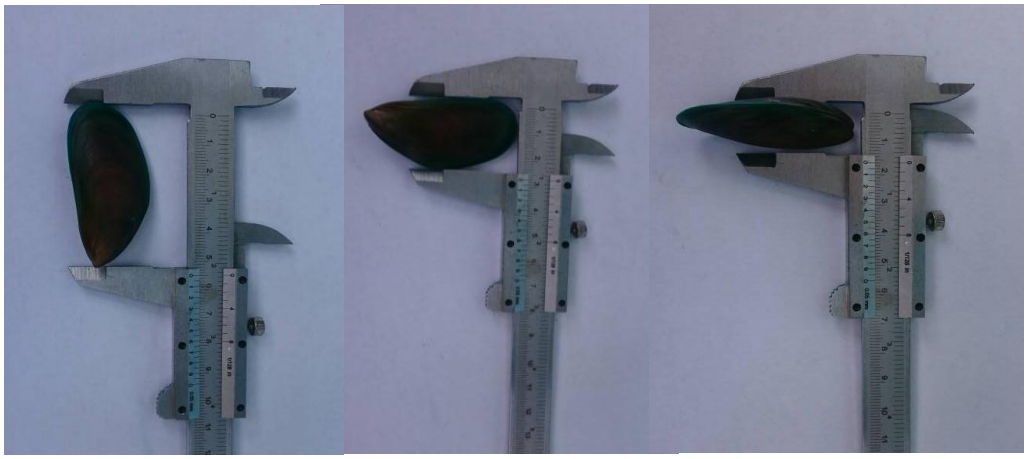

Figure 2. The measurement of *Perna viridis* dimension, length (a), height (b), and width (c).

Growth. The growth of mussels was evaluated using a manual ruler; the final dimension was measured from anterior to posterior of shells for every age in males and females (Sulvina et al 2015).

Shell length-height relationship. The allometric correlation between length and height was calculated by Vakily's (1989) formula, $W = aL^b$, where W is total weight (g) of mussels, L is total length (cm), and a, b are constant of linear regression.

Body condition index (BCI). The BCI was measured by calculating the dry weight of the soft tissue dried at 60°C until the weight remained constant divided by the dry weight of the shell (Huhn et al 2015).

Gonado somatic index (GSI) analysis. The gonads were separated from the mussel's meat and weighed, and the GSI analyzed was based on the histological characteristics of the gonads by using microscopical analysis.

Data analysis. The statistical analysis was conducted on ANOVA followed by Duncan's all-pairwise-comparison test using SPSS software version 2.40.

Results and Discussion. The *P. viridis* farming was carried out in Pasaran Island coastal waters since 2012 by using floating raft culture. The farming location is close to the Way Belau estuary and located in the intertidal zone with a water depth of about 8 m. The culture condition in Pasaran Island indicates an appropriate carrying capacity for *P. viridis* with salinity of 26-30 ppt, temperature 28-30^{o}C, pH 7.5, and dissolved oxygen 5.1-5.6 mg L^{-1}, brightness level ranging from 11.5-3.09 m, current 0.09-0.16 m s^{-1}, and the chlorophyll-a of the water were 10.83 mg m^{-3} (Ali et al 2015).

The cultivation process tends to be easy, including determining of the location, making a raft system, cultivation, and harvesting. The experimental site used floating raft placed around 200 meters from the coastline of Pulau Pasaran. A floating raft (10 x 10 m) can produce fresh *P. viridis* up to 3-4 tons, depending on the number of seeds, harvest age, and shell size (Noor 2015).

Determination of sexes and morphometrics. Even though sexual dimorphism in *P. viridis* is not quite evident, however, the results of the present study indicate several morphometric parameters that distinguish males and female and can be observed visually. Statistically, male and female *P. viridis* differed significantly ($p<0.05$) on the parameters of height, width, width ratio, weight ratio while the height ratio did not have significant effect ($p>0.05$) upon sexes (data was not shown).

Specifically, female mussels are longer but thinner than male shells at the same ages. This is in line with Villaluz et al (2016) that the differences in the body shape concerning sexes, male mussels appear to have a smaller shell size and wider width as

shown by the distance of their ligament region to its umbo. Whereas the female mussel has larger shell size, according to the distance from posterior adductor to its umbo region. Based on observations of the weight of female and male shells there is a tendency to have a relatively equal total weight, as well as meat weight, and total length of the shell ($p>0.05$).

Furthermore, observations based on the color of female tissues tend to be reddish orange while males are creamy towards orange chocolate (Figure 3).

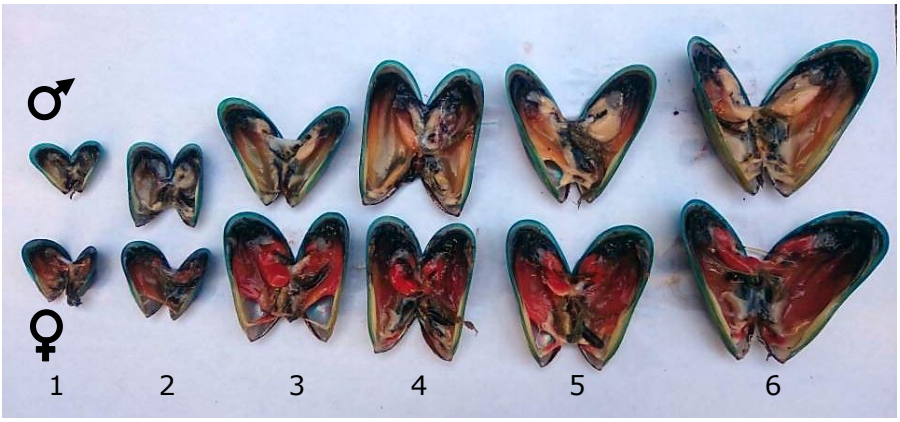

Figure 3. Tissues color of male and female *Perna viridis* at various ages (1-6 months). The color of female mussels is reddish while male are grey to orange-brownish (original).

According to this study, the determination of mussel's sexes can be performed from the age of four weeks of cultivation and more clearly during the longer cultivating period. Furthermore, the differences of beetwen sexes will be definitely clear at 12 weeks of cultivation were at the first time the female gonad appeared. It is quite difficult to differentiate sexes before the cultivation period reaches four weeks.

Arshad (2012) explained that the specific way in classifying female and male *P. viridis* is based on an internal morphology of the mantle and monitoring coloration of both male and female tissue, males are milky to creamy white while females are yellow-orange to dark-orange.

Growth rate. The total length of the *P. viridis* can reach 56 and 57 mm in male and female respectively after being cultivated for six months (Figure 4). The observations on age differences showed the increasing of mussel size during the prolonged period of cultivation ($p<0.05$), while sexes differences did not show significant values on the total length of mussel ($p>0.05$).

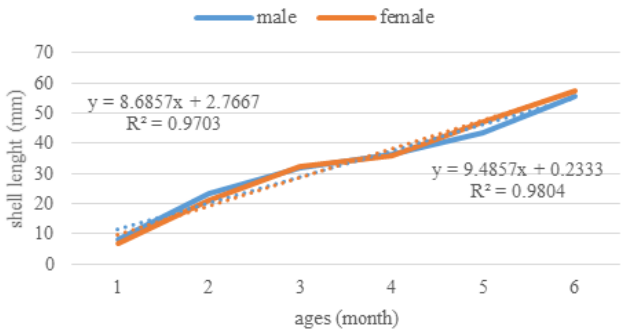

Figure 4. The growth rate of *Perna viridis*.

The growth rate of *P. viridis* cultivated in the Pasaran coastal waters is relatively high and reaches 9.3 mm mo^{-1}. Report from the Jakarta Bay shows an average monthly growth of 8 mm (Kastoro 1988), on the Philippine seashore can reach 10 mm mo^{-1} (Yap et al 1979), while the growth rate of *P. perna* in Brazil reached 8.6 mm mo^{-1} or able to reach 60 mm in seven months (Marques et al 1991). Urbano et al (2005), reported a smaller growth rate of *P. viridis* shell length only 7.1 mm mo^{-1}. The lowest growth rate in *P. viridis* was reported in Hong Kong waters, with a maximum growth rate of 5 mm mo^{-1} or only able to reach a size of 60 mm $year^{-1}$, due to the contaminated and unhealthy water in the cultivation area (Cheung 1993).

The tropical *P. viridis* farming has a harvesting phase commences when the mussels reach minimum commercial size. An optimal harvest of marketable size is achieved after a culture period of 6 months (Sivalingam 1977; Rivonker et al 1993), while Mohamed (2015), recorded the harvestable sizes reached within 4-6 months. The size of *P. viridis* in Pasaran island waters can reach 57 mm in six months cultivation. Rajagopal et al (1998) reported that *P. viridis* shows marketable size on 50-60 mm in length, achieved within a culture of 6 months. However, in subtropical mussel farming, the marketable size of *P. viridis* can be achieved only after 12-24 months cultivation period (Hickman 1992). This variation of mussel's growth rate is significantly influenced by species, geographic region, and cultivation method.

Shell length-height relationship. The relationship between the length and weight of male *P. viridis* were Y = 2.0072x-2.5338 while at females were Y = 1.7595x-1.921. By using these equation we obtained b values of 1.38 and 1.14 respectively (Table 1) which showed that the *P. viridis* type of growth was negative allometric, whereas the growth rate was more dominant than the weight.

Table 1

Constanta values of length-weight relationship on both male and female *P. viridis* from Pasaran Island coastal water, Lampung, Indonesia

Sexes	*Sample*	*A*	*B*	R^2
Male	180	0.18	1.38	0.8512
Female	180	0.15	1.14	0.8683

A, B = constant values, R^2= coefficient of correlation.

Differences in b constant value between males and females show differences in the growth rate of both sexes. Our finding are in accordance with Setyobudiandi (2000), male mussel grows faster than females showed by the greater b value of males compared to females (Rubianti 2001).

Body condition index (BCI). Differences of ages, sexes and both interactions have a significant effect ($p<0.05$) on the BCI values. The BCI value of male mussels in the early stage was higher than of females, although it was not significantly different. However, simultaneously overtaken from the third month, and in six months the BCI value of female have been twice greater than of males, 1.63 and 0.84 respectively (Figure 5).

It is showed that the nutrition of females is more intense than of males because of the possibility of gonad formation which in this study occurred three months after cultivation. According to Wang et al (2011), BCI values are in line with the nutritional status of *P. viridis*, the higher the BCI, the better the nutritional status of mussels. Meanwhile, Huhn et al (2015), explained that BCI is a positive linear function of phytoplankton abundance in the culture area.

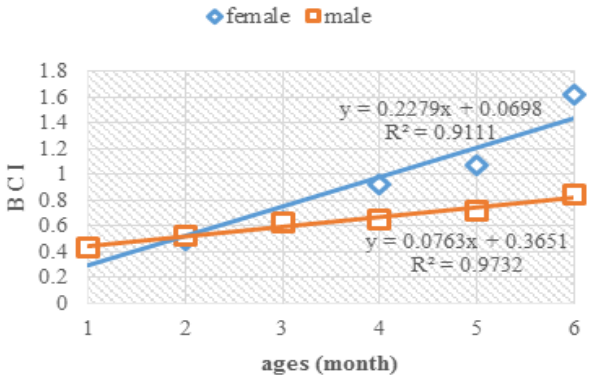

Figure 5. BCI's values of males and females of *Perna viridis* in different ages.

Gonado somatic index (GSI) analysis. The gonadal observation was performed three months after cultivation because this is the earliest time when gonads appear. It is proved by the fact that not all three-month-old mussels had gonad, whereas the 4, 5, 6 months old had. The three months gonad appeared tight and thickened and in the developmental phase, while on 4, 5 and 6 months are in the gonadal maturity phase seen from the increasing of gonadal size and widening cell wall.

According to McDonald et al (2018), there is a positive correlation between the *P. viridis* size and stage of reproductive development. The initiating gonad recorded tissue development from 6.5 mm in length. Shell length at three-months-old reached 32 mm and increased up to 57 mm at the age of six months of cultivation.

The three-months-old mussel is mostly light yellow in mantle pattern of developing gonadal tissue clearly visible which based on visual assessment score developed by McDonald et al (2018), belongs to stage 1. It is concluded to the early stage of gonad development, as shown, occurred of the primary oocyte by the tight and thickness of the cell walls (arrows) (Figure 6a). At the 4.5 and 6-months olds were in the phase of gonadal maturity seen from the increase in gonadal cell size and widening of the cell wall and the presence of vitellogenin and mature oocytes (Figure 6 b, c, d).

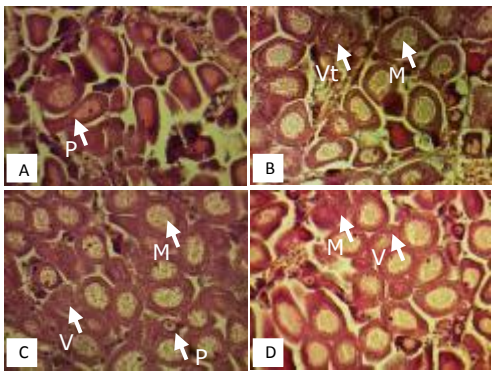

Figure 6. *Perna viridis* gonads observed at different ages 3, 4, 5, 6 months (a, b, c, d): PO - primary oocyte, V - vitellogenic stage, and M - mature stage.

Mantle color tends to be opaque with gonadal tissues, dark orange to brick red gonadal tissue (reproductively mature). McDonald et al (2018) stated that size and age have a positive correlation with gonad maturity. This finding also reinforces the information that the size of mature *P. viridis* is in the range of 30-35 mm (Kastoro 1988), while Siddall (1980), reported *P. viridis* becomes sexually mature at 15-30 mm in length, which equated to 2–3 months of age when is ready to spawn. Ompi & Svane (2018), states that

Mytilus galloprovincialis an outgroup of *P. viridis* can produce 7 million eggs in every spawning in accordance to Young et al (2002), marine invertebrates produce huge planktonic eggs which have a diameter fewer than 85 µm.

Conclusions. This study has successfully documented the baseline data of biological aspects of male and female *P. viridis* cultivated on raft culture. We found different values of BCI of males and females and also has different values in shell width, thickness, width ratio, weight ratio, while differences of ages of cultivation affected to the total weight, BCI values, total length, width ratio, weight ratio. The interaction of age and sexes affected the value of BCI. The older the age of the mussel, the BCI value will increase. BCI value in female mussels was twice greater than in the males, six months after cultivation. Gonads began to be found at the age of three months with gonadal maturity at 4-6 months of age.

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